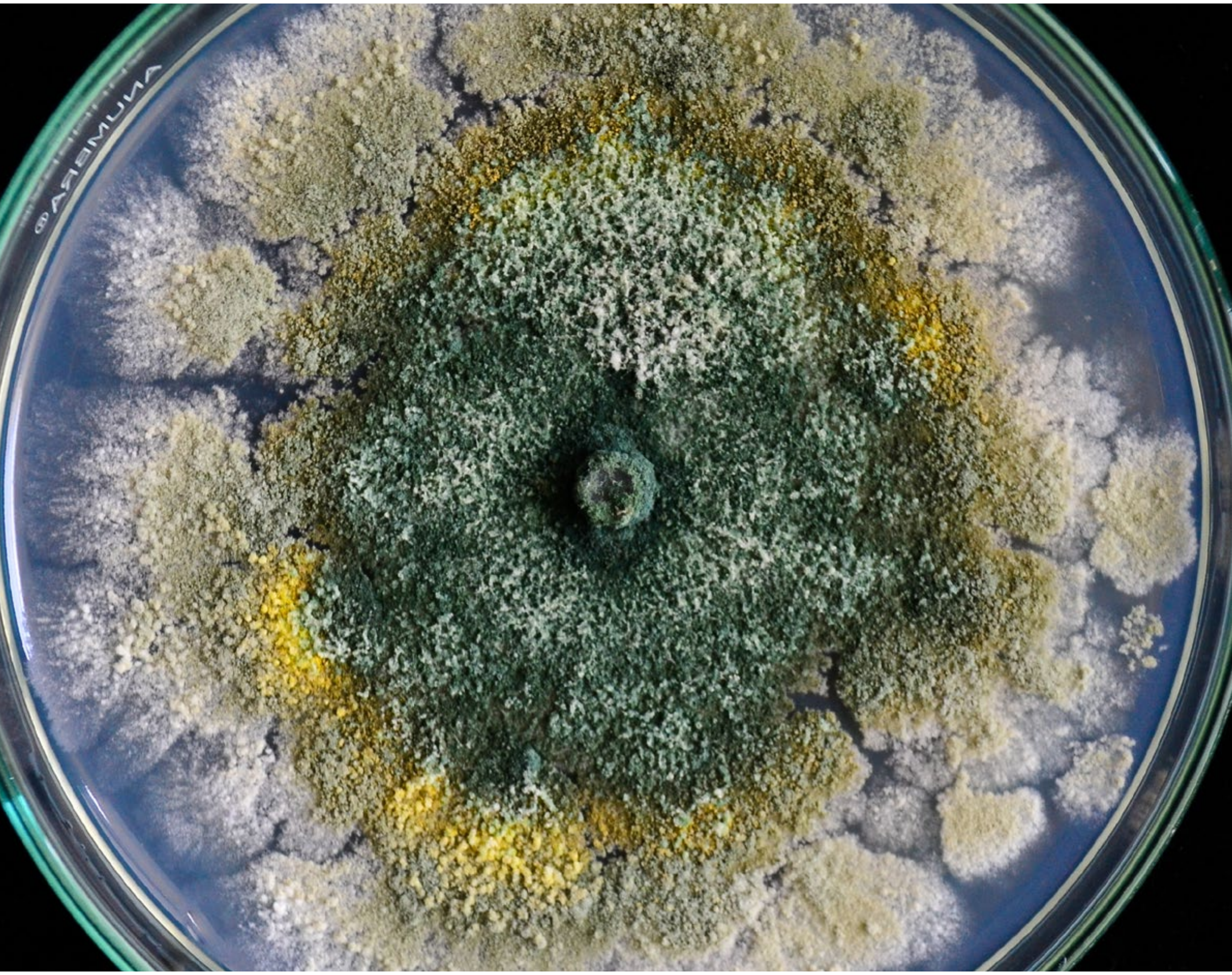

Agribusiness | Agrow

Bioformulations 2020

The Science, Technology and Business of Bioprotection Formulations

Please find the contents and sample pages below. If you have any questions, or would like to speak to a member of our team about this report please use our contact us page.

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Executive Summary

This report covers the main formulation aspects of the fast growing biopesticide and bioprotection market. Throughout the report, we examine the products and companies active in this market as well as the available patent and academic literature.

In Chapter One “Definitions and Market Basics”, we set the scene with definitions both by regulators where possible and other organisations who are active in the field. Specifically, we look at biostimulants, plant growth regulators, “low risk” plant protection products and initially the largest group of biopesticides. At the conclusion of the chapter, we look at some market figures which demonstrate why this sector is of such growing importance.

In Chapter Two “Main Categories of Biopesticides and Bio-Ag Ingredients and Key Features,” we look in more detail at the characteristics and key features of biopesticides and other bio-derived agricultural ingredients, and in particular how these relate to their formulation and application properties. The formulation properties of such ingredients are related less to their ultimate function than they are to their chemical and biological nature. Therefore, rather than dividing by application area, this chapter is divided into sections on (i) microorganisms, (ii) other organisms, (iii) peptides/proteins/enzymes and (iii) other naturally derived chemical ingredients.

In Chapter Three “Formulation Types – The Basics”, we look at the wide variety of formulation types available to the formulator of biopesticides and other bio-agricultural actives. In the main, these formulation types are the same ones available for the formulation of conventional synthetic pesticides. A huge selection of potential co-formulants and adjuvants is available so that the formulation can be given good stability, performance, application properties and usability. The specific manufacturing equipment which is required to produce the formulation will depend on the formulation type chosen as well as the desired properties of the final product. Specific factors which impact on the formulation of biopesticides and other bio-actives are also considered.

In Chapter Four “Formulation of Microorganisms for Agricultural Uses”, the key issues relating to formulating living microrganisms are considered with specific emphasis on viability/stability throughout the formulation process, storage, the application procedure and the field. The numbers of products in the different formulation types used for microrganisms both in solid and liquid form are presented graphically. A large section of the chapter deals with the most commonly used microbial biopesticide, namely *Bacillus thuringiensis*. Formulation aspects for fungi, viruses and bioinoculants are also considered.

In Chapter Five “Formulation of Enzymes and Peptides for Agricultural Uses”, we show that protein and peptide bioactives are currently in simple liquid and solid formulations. As the next generation of peptides to come to the market will consist of specially selected or designed active ingredients, we discuss more sophisticated formulation methods, such as microencapsulation of the active or immobilisation of the active on a carrier substrate which we expect to be required. To a significant extent the demands for proof of efficacy and stability will be driven by regulatory demands, as they are for conventional synthetic pesticide products. This will then drive the kinds of formulation approach needed. In this chapter we also discuss formulation aspects realting to a new category of actives used for bio-control treatment, namely RNA interference (RNAi).

In Chapter Six "Formulation of Other Natural Chemicals for Agricultural Uses", we cover the remaining natural extracts which can be chemically very diverse and complex. These extracts may range from unpurified and poorly characterised crude extracts to highly purified and well understood natural chemicals. Commercial products based on natural extracts are based on a relatively small number of active ingredients and just a few different main formulation types. Oil-based actives (e.g. neem, terpenes, pyrethrins) are formulated mainly as emulsifiable concentrates and water-soluble actives (e.g. fatty acid salts, saccharides) are formulated as water-based soluble liquid concentrates. Most commercial formulations are liquids with just a few granules and seemingly only few of the more sophisticated formulation types (microemulsions, microcapsules) are seen on the market. As far as can be determined, many of the co-formulants used in these formulations are synthetic (e.g. conventional surfactants, petroleum-based solvents). If more "all-natural" formulations are demanded in the market, then there will be scope for naturally derived co-formulants to fill the gap.

In Chapter Seven "Formulation of Other Bio Ingredients for Agricultural Uses", formulation aspects relating to macrorganisms such as insects and nematodes as well as pheromones are considered. For insects, the carrier materials and packaging types are discussed. For nematodes, the main approaches considered are the use of carriers and encapsulation in matrices such as alginates. In addition, the use of insect cadavers as carriers for nematodes is discussed. For pheromones, the emphasis is on techniques used for controlled and sustained release.

In Chapter Eight "Company Review" we summarise the main formulation activities of companies in four categories: (i) the traditional agrochemical majors; (ii) smaller companies and biocontrol/biostimulant specialists; (iii) start-ups and (iv) supply chain companies. The major agrochemical companies have begun to move into biocontrol and other biological products. Underpinned by financial strength, good market access and extensive R&D resources, we expect the majors to grow their position in biological product formulation. The main base of the biological market has historically been smaller companies. These companies show signs of formulation innovation although most formulations on the market are still conventional. In the formulation supply chain, both contract manufacturers and co-formulant suppliers present a mixed picture. Some are visibly active in the biopesticide and biostimulant markets, but many are not yet promoting their capabilities in this area.

In Chapter Nine "Future Directions", we discuss and speculate about future trends and drivers in this market. Amongst the topics discussed are "Smart Agriculture", regulations, mixtures and combination treatments, new opportunities, co-formulants, inerts, and adjuvants. Future market activity including mergers and acquisitions are also discussed. We conclude that formulation will be key to meeting growth expectations and will follow mainly the same path as that for conventional plant protection products with the exception that the changes are likely to happen much more quickly.

Table 3: Plant Processes and Main Classes of PGR (Adapted from Canna[xxi])

	Germination	Growth to maturity	Flowering	Fruit Development	Seed Dormancy	Abscission
Gibberelin	→	→	→	→		
Auxin		→	→	→		
Cytokinin		→	→	→		
Ethylene			→	→		→
Abscisic acid					→	→

Gibberelins are partly responsible for cell division and the elongation of stems and other tissues. They help to stimulate flowering within plants and can play a role in root formation. Auxins are responsible for plant cell elongation and have an effect on rooting. Cytokinins can be used to stimulate or retard plant growth and are sometimes used as antagonists for auxins. Ethylene influences root development and shoot growth whilst abscisic acid dictates germination and water stress management[xxii].

1.4 Low Risk Plant Protection Products

As well as biopesticides and organic farming, there is a class of registered actives which the EU has defined as being low-risk plant protection products and stated that these should be preferred by farmers. The development and placing on the market of these substances is encouraged by the substances being approved for 15 years rather than 10 and data protection on submitted studies being prolonged from 10 to 13 years. They also claim that a fast track authorisation procedure with reduced timelines (120 days instead of one year) means that these products can be placed on the market quickly and that the low risk status can be used in advertising.

A search on the EU Pesticides Database[xxiii] in December 2018 showed that only 13 actives (up from 11 in December 2017) were listed as low risk active substances. The list from December 2018 included five fungicides, four elicitors, one insecticide, one molluscicide and one plant activator. Elicitors are compounds which activate the chemical defences in plants.

The full list of approved low-risk active substances from December 2018 is detailed in Table 4 and a brief discussion of these is now provided

Table 4: Low-Risk Active Substances Approved by the EU

Substance	Active Substance Id	Category	Approval Date	Approval Expiration
Bacillus amyloliquefaciens strain FZB24	2324	FU	01/06/2017	01/06/2032
Cerevisane	2301	PA	23/04/2015	23/04/2030
Coniothyrium Minitans Strain CON/M/91-08 (DSM 9660)	1156	FU	01/08/2017	31/07/2032
COS-OGA	2313	FU	22/04/2015	22/04/2030
Ferric phosphate	1362	MO	01/01/2016	31/12/2030

Figure 8: Schematic showing the process of phage infection and replication

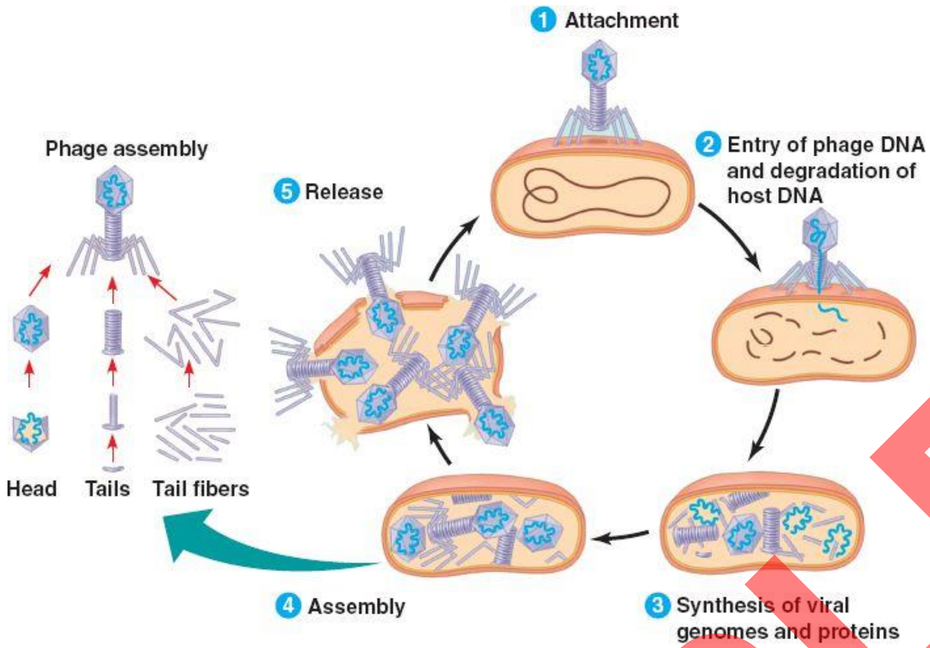

Image: *https://ww2w.flickr.com/photos/hixtine/6374709127*

Viruses and Phages: Characteristics of Importance to the Formulator

If the biological product to be formulated is based on a phage then essentially the product will be a formulated bacterial product in which the host bacterial cells are infected with the virus. In this case the same considerations will apply as described in the section above on bacteria.

Viruses can also be formulated on the basis of aqueous suspensions or dried viral material, i.e. in the absence of a host organism. As with other biological active ingredients, freeze drying is one common method of producing stable dried material[lii]. The formulator of viruses should take the following factors into account:

- Influence of heat, cold or freeze-drying process on the virus, particularly with respect to potential denaturing of the DNA or RNA material;
- Potential chemical interactions of excipients with the virus and the effect of pH. DNA or RNA material may be sensitive to degradation e.g. by oxidizing or reducing agents or at low pH;
- The potential of shear or other processing steps such as filtration to damage the three-dimensional structure of DNA/RNA or protein material in the virus;
- The size of the virus particles if a filtration step is being used.

The most common formulation types in this category are:

DP: Dustable Powder. These are cost-effective and relatively simple formulations which are typically blended powders consisting of an active ingredient and an inert filler. The active ingredient may need to be dry-milled to a fine particle size before blending and diluting with filler. As with WP formulations the main area for biopesticide formulators to consider are the risk of heat or shear damage on dry milling.

GR: Granule. In contrast to WG formulations, dry application granules (GR) do not have to be water dispersible. In addition to the active ingredient, carriers and fillers may be used. Carriers can be organic or inorganic and are often used when the active ingredient is a liquid to absorb the active and produce a dry material for formulation as a solid. GR formulations will be less dusty than DP formulations and will have better flow properties. GR products are frequently produced by extrusion granulation in the same way as for WG. As with WG, the main concerns for formulators of biopesticides to address relate to the processing conditions, which can involve heat and shear.

DT: Tablet for Direct Application and TB: Tablet. Tablet formulations are used where a controlled dosage is required. Tablets may contain the active ingredient, binders, fillers, flow aids, lubricants, dispersing agents and disintegrants. Typically, ingredients are dry-blended before compression in a tablet press. Again, robustness of bio-actives to the processing conditions is the most important area for formulators to consider.

Less common formulation types in this category include:

BR: Briquette. Solid block designed for controlled release of the active ingredient, e.g. into water. The co-formulants used are likely to be similar to those used for tablets.

CP: Contact Powder. Powder for direct application to target, e.g. for insecticides or rodenticides.

MR: Matrix Release. The active ingredient is trapped within a polymer matrix and is then released slowly when in use.

PR: Plant Rodlet. A rodlet is several cm in length and several mm in diameter. It may be a more convenient solid form than GR or DT/TB. The co-formulants used are likely to be similar to those used for tablets.

3.2.3. Formulations for Seed Coatings

As the name implies, seed coatings are liquid formulations which are applied to seeds. The coating is then dried before the seed is planted. The active ingredient can then work before, during and after germination. Potentially this more targeted application method can lead to a lower pesticide load than for conventional spraying. Biopesticides and other bio-actives can be applied as seed treatments, as can conventional synthetic chemical pesticides.

There are several different types of seed coating formulations, most of which are analogous to the formulation types described above. In general, the comments made above relating to biopesticides and other bio-actives will also apply to the equivalent seed treatment formulations. In brief these seed treatment types are:

DS: Dry Powder for Seed Treatment. These products resemble DP formulations but may additionally contain stickers (usually oils) which can assist adhesion to the target. DP products are simply dry-mixed with the seeds before packing in the following sections of this chapter. We can speculate, however, that the encapsulation may bring some stability benefits.

An important property of any insecticide is the stability of the product in the field and one of the weaknesses of Bt is its tendency to degrade in the presence of UV light. This has been well-known for some time and in 1991, Pusztai *et al* presented data supporting a photosensitization mechanism involving the presence of exogenous (and possible endogenous) chromophores which create singlet (and highly reactive) oxygen species upon irradiation by light[lxxxi]. It is worth noting that one of the products cited in the BCPC Biocontrol Manual specifically mentions the addition of a built-in UV Protectant.

BioDalia's Bio T Plus[lxxxii] is a liquid concentrate of Bt subsp. *kurstaki* which claims a two-year shelf life at room temperature and a relatively long persistence in the field due to a highly effective formulation containing the UV protectants, a surfactant adjuvant and bait. Examination of the Safety Data Sheet suggests it is in essence an SC formulation with 84% water. Inert fermentation remains are 6.8% and preservatives are added at 1.0%. The "formulation" is 7% which we can assume are the surfactant and UV protectant and the remainder is the active ingredient.

In the section reviewing literature and patents, we discuss the options for enhancing the UV stability of Bt and other microbial formulations in the field.

4.4 Other Bacteria used as Biopesticides

As well as the widely used Bt, there are a number of other bacteria used commercially as biopesticides. These include *Burkholderia*, *Saccharopolyspora spinosa*, and *Bacillus firmus*. In Table 9 we look at a selection of these commercial products and the formulation types compiled from the University of Hertfordshire Database, company websites and the review article published in 2018 by Luca Ruiu[lxxxiii].

As we have seen previously, the majority of the formulations are relatively simple suspensions or powders/granules. The trend towards combining biopesticides with conventional pesticides as observed with the Poncho®/Votivo® and REGEV™ products is one which will be discussed later in this report.

Table 9: A Selection of other Bacteria used as Biopesticides

Species	Product	Manufacturer	Formulation
Heat killed *Burkholderia* spp *strain A396*	Majestene®	Marrone Bio Innovations	Likely SC
Heat killed *Burkholderia* spp *strain A396*	Venerate® XC	Marrone Bio Innovations	Likely SC
Saccharopolyspora spinosa	Tracer™ 120	BASF	SC
Saccharopolyspora spinosa	Conserve™	Corteva	SC
Chromobacterium subtsugae PRAA4-1	Grandevo® WDG	Marrone Bio Innovations	WDG
Bacillus firmus	BioNemaGon™	AgriLife	WP
Bacillus firmus I-582	Chancellor	Agrogreen Biological Division	Liquid

Plant Pests". In the patent they describe Suspension Concentrates which are stable over two weeks of storage at elevated temperature. The formulations shown in Table 11 are not very different from those used for conventional pesticides and further examples of suspension concentrates used for seed coating again use common co-formulants such as xanthan gum as a thickener and standard preservatives such as 1,2-benzisothiazolin-3-one (BIT) and 2-methyl-4-isothiazolin-3-one (MIT).

Table 12: Suspension Concentrate Formulations for Example 18[xciv]

Component	Function	18A %(w/w)	18B %(w/w)
RTI $3x10^{11}$cfu/g	Active Ingredient	5.25	9.59
Ammonium Sulfate	Antifreeze	9.5	
Glycerol (86.5%)	Antifreeze		48
Attapulgite (25-30% aq. Suspension)	Thickener	9.0	
Alkyl polyglycosides, mixture	Dispersant	8.0	
Anionic Phosphate ester surfactants, mixture	Dispersant	7.0	
Ethylene Vinyl Acetate Dispersion	Dispersant		0.89
Silicone Emulsion	Antifoam	0.3	
Potassium Sorbate	Preservative	0.1	0.2
Water	Dilutent	59.54	38.82

Moving on to soil inoculants, a patent from Valent Biosciences specifically claims a formulation of mycorrhizal seed and in-furrow compositions containing soybean oil[xcv]. Excipients used are emulsifiers, a rheological additive, a polar additive and a dispersing agent. Non-ionic emulsifiers are preferred and once again Tween® 20 is preferred at a level of 0.5 to 4% w/w together with Atplus® 300FA, once again from Croda and at a level of 2.5% to 7.5% w/w. Rheological additives suitable for use are organic derivatives of clay such as the Bentone® range from Elementis and Garamite® 1958 from BYK Additives Inc. Polar additives suitable for use include Jeffsol® AG 1555 from Huntsman Corporation and a very wide range of dispersants are claimed such as Methylcellulose and Ethylene Oxide-Propylene Oxide block polymers (EO/PO). There are many formulation details in the patent and the most preferred embodiment is a seed treatment composition comprising:

- 8.7% w/w Mycorrhizae technical powder concentrate
- 82% w/w soybean oil
- 5% w/w of a surfactant comprising polyol fatty acid esters and polyethoxylated derivatives thereof
- 2% w/w benzenemethanaminium, N,N-dimethyl-N-octadecyl-,chloride, reaction products with hectorite
- 2% w/w 1,2-propanediol cyclic carbonate
- 0.5% w/w polysorbate 20.

field trials, so the article is unusual in referring in some detail to formulation and stability aspects for these materials. The formulations appear to be relatively simple solutions but in concentrated solutions the peptides described can aggregate over time and lose some of their activity. In this example, water miscible organic solvents were used (especially ethanol) as well as acid. The use of these allowed more concentrated formulations to be produced without loss of activity. The authors also observed that calcium chloride could act to reduce the activity of the formulation but the chelating agent EDTA could be used in the formulation to increase the activity of the formulated peptide. However, the addition of sugars to the formulation was generally detrimental to the activity of the peptides[cvi].

Although these findings relate to the specific peptides tested, it is to be expected that the challenges of aggregation and interactions with salts and solvents will be common to most peptide formulations of this type.

5.2.4. NanoNema Project (Portugal)

NanoNema is a research project funded by the European Union and Portuguese authorities[cvii]. The objective of the project is to develop a biodegradable nanoparticle formulation. The particles act as carriers for the peptide active ingredients and are intended to improve product handling and bioavailability. The nanoparticles will be produced from gelatine and polylactic acid from the fermentation of whey lactose.

5.2.5. University of Queensland (Australia) and University of Cambridge (UK)

In a publication which described the spray application of RNAi actives to treat plants against transmission of plant viruses via aphids, formulation issues are mentioned[cviii]. In particular the stability of the dsRNA formulation was improved by formulating with layered double hydroxide (LDH) nanoparticles in a ratio of 1:2 dsRNA:LDH. Thus, the LDH appears to act as a substrate or carrier on which the dsRNA is deposited. An earlier publication describes how the LDH formulation can improve resistance to washing off as well as a sustained release effect[cix]. The University of Queensland has named the product BioClay and has partnered with NuFarm although to date BioClay is not yet on the market[cx].

5.2.6. OECD Conference on RNAi Based Pesticides – 2019

A number of the presentations from this recent conference[cxi] touched upon the importance formulation to RNAi based pesticides. In brief, the following are noteworthy:

- Formulation can improve delivery of RNAi insecticides (S. Whyard, University of Manitoba);
- Formulations can improve uptake of dsRNA in insects (O. Christiaens, Ghent University);
- RNAi normally degrades rather readily. However, risk assessments of RNAi products may need to take into account the effects of formulations such as encapsulation which may reduce the tendency to degrade (A. Gathmann, German Federal Office of Consumer Protection and Food Safety);
- Risks associated with carriers used to formulate and deliver dsRNA will need to be assessed on a case-by-case basis (N. Mitter, Centre for Horticultural Science, Australia).

Chapter 6: Formulation of Other Natural Chemicals for Agricultural Uses

6.1 Other Natural Chemicals: Overview

In chapter five we have covered the formulation of one category of natural biochemicals, namely proteins and peptides. This chapter will cover remaining natural extracts which can be chemically very diverse and complex. These extracts may range from unpurified and poorly characterised crude extracts to highly purified and well understood natural chemicals. The source of these extracts is diverse – they may derive from plants, microorganisms or even from animals. Water soluble extracts are extracted as aqueous solutions which can be formulated as solutions or dried before formulation. Solvent/oil soluble extracts can be extracted as non-aqueous solutions which can then be emulsified in formulation or alternatively the solvent can be removed first. Supercritical fluids such as supercritical CO_2 can also be used for extraction to yield a dry active ingredient. As active ingredients, their function in agricultural usage is equally diverse and they can be used as herbicides, fungicides, insecticides, semiochemicals, biostimulants etc[cxlv cxlvi]. Likewise, the application method can also vary and can include spraying, seed treatments etc.

Chemically speaking this category of extracts can vary widely and can includes common types such as oils, fatty acids, polysaccharides, oligosaccharides and lignins. This means that the physical properties can also vary widely (see also section 2.4 of this report). We can summarise the key features of this category of actives as:

- They are often poorly characterised mixtures of related or unrelated molecules, the composition of which may vary according to origin, season, climate etc;
- They may be small molecules, oligomers or polymers;
- They have a wide range of water solubility from insoluble oils through to highly soluble saccharides;
- They are likely to be sensitive to heat, light, shear, pH and other chemicals.

Natural extracts can be formulated as any of the main formulation types (see chapter three of this report) and this list also represents the main factors which formulators have to take into account when developing a formulated product. As with other categories of product the main concerns of the formulator will be:

- Quality and reproducibility of the active ingredient (or extract mixture);
- Stability of the product;
- Maintaining or enhancing efficacy of the active ingredient in the application;
- Compatibility of the product with packaging, application equipment and other products such as adjuvants;
- Manufacturability of the product and manufacturing cost.

- Stimuter is a root-stimulant for potatoes and - like the above products - is a concentrated water-based formulation based on herbal concentrates;
- Tercol and Nemater are further root stimulants, again based on plant extracts. The products are available as liquid, granule or micro-granule formulations;
- Gramisec is a growth stimulant product for turf applications based on plant extracts. Liquid formulations are applied by spraying and the granular formulation is applied in soil before sowing.

6.4.28. Seipasa[cxcvi]

Seipasa (Spain) supplies biocontrol and biostimulant products.

- Pirecris® is an insecticide based on pyrethrum extract. AI content is 20g/L and the formulation type is an emulsifiable concentrate (EC). The formulation is patented (see patent section 6.3).
- Nakar™ is a further insecticide, this time based on plant derived oleins which have been saponified. The active ingredient is therefore a fatty acid variant. The formulation is a water-based soluble liquid concentrate (SL) with 40.8% w/w fatty acid.
- Sweetsei™ is sold to assist the ripening of fruit. It includes biological components and an organic polymer.

6.4.29. Servalesa[cxcvii]

Servalesa (Spain) supplies biocontrol and biostimulant products.

- Triac® is an emulsifiable concentrate (EC) bioinsecticide formulation with natural pyrethrum (4.0%) as the active ingredient;
- Miles® is used to strengthen the defences of plant against fungi and other pathogens. The active ingredient is a natural extract of *Equisetum arvense* L (2.0%) in a liquid formulation;
- Gamasystem S® is a biostimulant based on chitosan (extracted from crustaceans). The product is a 2.5% aqueous solution formulation;
- Biocrop® is a biostimulant containing various components extracted from algae. The total active content is 3.5% and is an aqueous solution formulation;
- Further biostimulants based on the same algal extract (all liquid solution formulations) include Biocuaje®, Biocuaje ECO®, Bioengorde®, Olivo Plus® and Olivo Plus® B1. The main organic components are alginic acid and mannitol;

In the patent RU2225107C2 “Method for Preparing Preparative Form for Storage of Entomopathogenic Nematodes of families Steinernematidae and Heterorhabditdae” improved storage is claimed by dispersing nematodes into sodium alginate and Tween®80 and then adding the perflurorocarbon-ethyl ester of 2-trifluoromethyl-3-oxoperfluorohexanoic acid. The perfluorocarbon is proposed to supply a source of dissolved oxygen enabling the nematodes to survive for a longer period. The solution is then subsequently mixed with a hydrophilic silica filler (BS-100). The subsequent powder is then placed in polyethylene bags and stored at a temperature of 2-22˚C.

In KR100515009B1 entitled “Formulation Method for Prolonged Storage and Efficiently use of Entomopathogenic Nematodes at Powder Condition” and assigned to Bicosys in Korea, a powder formulation of EPNs containing sorbitan monooleate (Span®80), polyoxyethylene silica, sawdust powder, glycerol, Keltrol F, and trehalose bound to conventional materials such as decyl monoether (PLE 3) and sodium silicate is claimed to improve storage and efficacy.

Further research into this “conventional” formulation approach to improving the shelf-life of EPNs is still ongoing as is demonstrated by the recent patent CN109845730 entitled “Powder Containing Insect Pathogenic Nematodes” and assigned to the University Hebei Agriculture in China. The claims are related to a powder comprising a substrate, a water-retaining agent, an ultraviolet protective agent and a preservative. The powder includes glycerine and water and the substrates preferred are silica and/or clay. The water retaining agent preferred is hydroxypropyl methylcellulose and/or sodium carboxymethylcellulose and the preferred UV protectant is sodium alginate and/or xanthan gum. These latter compounds are not normally used for this purpose in formulations, but the patent does detail formulations and processes including these compounds. Data demonstrating that they are actually absorbing UV is not provided. In a further specific embodiment, the preservative is claimed to be potassium sorbate and/or formaldehyde. It is unlikely that formaldehyde will be acceptable to the wider market but potassium sorbate and other similar preservatives from the food area could be possible.

A relatively straightforward approach to extending shelf-life of EPNs is contained in KR20150001295A “Formulation Method for Entomopathogenic Nematodes and Formulation prepared therefrom” assigned to Ecowin Co Ltd in Korea. The improvement in shelf life is claimed to be down to a process which includes a surface sterilisation step. More specifically, the method for formulating entomopathogenic nematodes comprises the steps of:

a) soaking entomopathogenic nematodes in a sodium hypochlorite solution and sterilizing the surface;

b) washing the surface-sterilized entomopathogenic nematodes with distilled water;

c) putting the surface-sterilized and washed entomopathogenic nematodes into a glycerin solution and culturing to induce to a dormant state; and

d) mixing the glycerin solution containing the entomopathogenic nematodes induced to the dormant state to a cellulose powder.

The approach of encapsulating EPNs in alginates is well documented and was first demonstrated in the scientific literature in 1985 by Kaya and Nelsen[ccxix]. The approach was also the subject of US patent 4615883 entitled “Hydrogel encapsulated nematodes” assigned to Plant Genetics Inc. This describes a formulation of *biological and chemical compounds. This means farmers can have a well-protected and strengthened crop right from the beginning of its life*".

BASF's biological products are principally based on microorganisms (bacteria and fungi) as well as beneficial nematodes[cclxviii]. The latter products are a consequence of BASF's acquisition of Becker Underwood in 2012 which also included certain biostimulants[cclxix]. As we have seen in chapter 4, BASF's microorganisms are principally formulated as suspension concentrates. BASF also distributes STK's Timorex Gold (EC formulation of teat tree oil extract) in Brazil (see chapter 6). R&D into sprayable RNAi products is also ongoing (see chapter 5).

BASF recently acquired from Bayer a seed business which includes products such as Poncho Votivo[cclxx] - a product containing a bacterial strain which is active against harmful nematodes.

BASF maintains a significant resource in formulation knowhow for its Crop Protection division at its R&D centres in Germany, USA and India.

While the number of biocontrol products remains relatively small[cclxxi], BASF' statements of intent in this area, supported by its strong formulation expertise, will mean that it is likely to be a significant player in this area in future years.

8.2.2. Bayer Crop Science

Bayer's Crop Science business is one of the world's largest multinational suppliers of crop protection products. Although most of these products are conventional chemical pesticides, there are a number of biocontrol products in its range. Formulations (both EC and SL) based on natural extracts are detailed in chapter 6. In chapter 4, biocontrol products based on microorganisms are described, these include both liquids and solids (WP and GR). Bayer are also active in sprayable RNAi products, some of this activity derives from the acquisition of Monsanto (for further detail see chapter 5).

Bayer remains active in formulation R&D (e.g. with numerous patent applications) to support its Crop Science business, with significant expertise centres in formulation in Germany and USA.

8.2.3. Corteva

Corteva resulted from the merger of the former Dow and Dupont agrochemical businesses and is one of the largest global suppliers of conventional synthetic agrochemicals. Its new fungicidal active (fenpicoxamid) is a derivative of a bacterial extract and is co-formulated with a synthetic active (see chapter 6). In chapter 4 we provided details of a microbial pesticide formulated as SC but overall Corteva has less activity in the biological area than other major crop protection companies[cclxxi]. They do maintain formulation expertise for biologicals in their US R&D centre, however.

8.2.4. Syngenta

Although Syngenta is one of the world's leading suppliers of conventional synthetic pesticides, it also offers some biocontrol products. In chapter 6 we detailed several formulations based on natural extracts, including Timorex Gold® licensed from STK in some territories. There is little sign of current activity with peptide